

AMENDMENTS TO THE SPECIFICATION:

Please insert the following paragraph at page 1 after the title:

This application is a divisional application of Serial No. 09/598,216, filed June 21, 2000, now U.S. Patent No. 6,486,123.

Please replace the paragraph beginning "Escherichia coli" at page 17, line 15, with the following amended paragraph:

Using the Escherichia coli expression system described by Shen, et al. (1993); U.S. Patent No. 5,753,465; and Kim, et al. (1995); U.S. Patent No. 5,843,888, new non-naturally occurring artificial recombinant hemoglobins ("rHbs") have been constructed, having low oxygen affinity while maintaining high cooperativity in oxygen binding. One of the rHbs, rHb ( $\beta$ N108Q) also exhibits increased resistance to autoxidation as compared to certain other known low oxygen affinity mutants. More particularly, the present invention is directed to: a recombinantly produced mutant of Hb A, denoted herein as rHb ( $\beta$ N108Q), in which the asparagine residues at position 108 of each of the  $\beta$ -chains (SEQ ID NO: 8), located in the  $\alpha_1\beta_1$  subunit interface and in the central cavity of the Hb molecule, have been replaced by glutamine residue; and a recombinantly produced mutant of Hb A, denoted herein as rHb ( $\beta$ L105W) in which the

leucine residues at position 105 of each of the  $\beta$  chains (SEQ ID NO: 8) have been replaced by tryptophan and in this molecule a new hydrogen bond is formed from  $\beta 105\text{Trp}$  to  $\beta 94\text{Asp}$  in the  $\alpha_1\beta_2$  subunit interface in order to lower the oxygen binding affinity by stabilizing its deoxy quaternary structure.

Please replace the paragraph beginning "The construction of plasmid pHE7009" at page 25, line 5, with the following amended paragraph:

The construction of plasmid pHE7009 for expression of mutant rHb ( $\beta\text{N108Q}$ ) using the human globin cDNAs was carried out as follows. The coding sequences of human  $\alpha$ - and  $\beta$ -globin cDNAs in plasmid pHE7 were inserted into pTZ18U (Bio-Rad Laboratories, Hercules, CA) by methods well known in the art. Site-directed mutagenesis was performed as described by Kunkel, T.M. et al., Proc. Natl. Acad. Sci. USA 82:488 (1985) the disclosures of which are incorporated herein by reference, and Shen, et al. (1993). An oligonucleotide of sequence 5'-ACAGACCAGTACTTGTCCCAGGAGCCT-3' (SEQ ID NO: 4) (mutated codon Asn $\rightarrow$ Gln is underlined) was purchased from DNA International, Inc. (Lake Oswego, Oregon), and used as the mutation primer. The human normal  $\beta$ -globin cDNA in plasmid pHE7 was then replaced with the mutated cDNA to produce plasmid pHE7009. The DNA sequences for the  $\alpha$ - and  $\beta$ -globin cDNAs in pHE7009 are shown in Figure 1A (SEQ ID NO: 5). The amino acid

sequence for the human beta chains of hemoglobin is shown in SEQ ID NO: 8. Plasmid pHE7009 in host cell E. coli JM109 and designated pHE7009/JM109 was deposited with the American Type Culture Collection of Manassas, VA on April 27, 2000 under number PTA-1768.

Please replace the paragraph beginning "The construction of plasmid pHE7004" at page 25, line 20 with the following amended paragraph:

The construction of plasmid pHE7004 for expression of mutant rHb ( $\beta$ L105W) using the human globin cDNAs was carried out in the similar way as that of plasmid pHE7009, except an oligonucleotide of sequence 5'-CCTGAGAACTTCAGGTGGCTAGGCAACG TGCTGGTC-3' ((SEQ ID NO: 6), mutated codon Leu→Trp is underlined) was purchased from DNA International, Inc. (Lake Oswego, Oregon) and used as the mutation primer. The DNA sequences of the  $\alpha$ - and  $\beta$ -globin cDNAs in pHE7004 are shown in Figure 1B (SEQ ID NO: 7). The amino acid sequence for the human beta chains of hemoglobin is shown in SEQ ID NO: 8. Plasmid pHE7004 in host cell E. coli JM109 and designated pHE7004/JM109 was deposited with the American Type Culture Collection of Manassas, VA on April 27, 2000 under number PTA-1769.

Please replace the previously filed Sequence Listing with the supplemental Sequence Listing filed herewith.